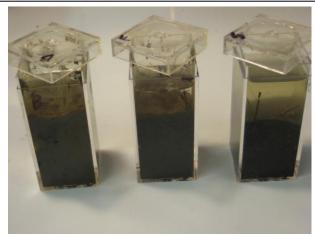
#### Science Lesson Plan:

## **How to Make a Microbial Mat**

Brad Bebout, Allison Pfeifer, Erich Fleming, Adrienne Frisbee, Jan Winter NASA Ames Research Center

# **Background**

Microbes have shaped the atmosphere and climate of Earth over geologic time. Microbes may be important in the search for life both within our solar system and on extrasolar planets. Some of the earliest and most widespread evidence of life on Earth were in the form of microbial mats. Microbial mats are complex, usually layered collections of microorganisms that can be observed in a number of aquatic ecosystems. They are complete ecosystems in miniature. In this activity, students create living versions of the microbial mats that have existed on earth for 3.5 billion years.



Living microbial mats constructed in plastic boxes.

## **Main Concepts**

Photosynthetic microbial mats are complex ecosystems that generally exhibit vertical gradients in light, and chemical compounds. These gradients are established by photosynthesis at the top of the mat and control the functioning of the intact ecosystem.

#### **Scientific Question**

What factors contribute to the vertical structure of microbial mats in the field?

# **Objectives**

- 1. The students will create their own microbial mat.
- 2. The microbial mat will be available for later experimentation and/or observation.

#### **Abstract of Lesson**

Microbial mats are defined as layered communities of microorganisms that are only a few millimeters thick. Many biogeochemical processes are conducted by the organisms in the mat to sustain life. For example, the mats perform photosynthesis, and respiration, and can acquire nitrogen through the process of nitrogen fixation. The mat community is a fully functioning ecosystem containing both producers and consumers or organic carbon. The upper layers of the mat contain cyanobacteria that create their own food (organic carbon) through the process of photosynthesis. Light energy is used to split electrons from water and the energy carried by these electrons is used to reduce carbon dioxide

into sugars, which are, in turn, used by the cyanobacteria for food. In the process, cyanobacteria in the surface (sunlit) layer also produce oxygen. Below this layer, other bacteria that are tolerant of oxygen, utilize the energy liberated by combining the sugars with oxygen for growth. At the bottom layer of the mat, where oxygen does not penetrate, other microbes, which can combine the organic matter with chemicals other than oxygen, may be found. These microbes utilize a number of chemicals other than oxygen to oxidize the organic matter left behind by oxygen utilizing microbes. These chemicals include nitrate, manganese, iron, and sulfate. Many of these microbes actually cannot tolerate the presence of oxygen.

In this exercise, we will use live microbes, and inorganic materials, like sand, to construct a type of "microbial mat". The "mats" that we make might not ever be found in nature, but will resemble natural mats closely, and will function like microbial mats known from a number of extreme environments. The mats we make here can be used for further experiments that will demonstrate a number of important principles in biogeochemistry and microbial ecology

# **Prerequisite Concepts**

Students should have general familiarity with laboratory work.

## **Major Concepts**

- 1. Microbial mats are complicated microbial ecosystems.
- 2. Microbial mats display vertical structure (e.g., they have oxygen available at the top of the mat, but not the bottom).
- 3. The microorganisms in mats both orient themselves to vertical gradients and contribute to making the gradients as a result of their metabolism.

#### Standards Met:

Project 2061 AAAS 1993: 5.D.1

5.F.8 5.F.9

### **Materials List:**

- 1. Clear plastic container. You will at least one per student. We often use small plastic boxes that we get from a local plastics store or a container store.
- 2. Paper for carbon source (laboratory Kimwipes or unbleached paper towels)
- 3. Plaster of Paris as a source of sulfate (the yolk of a hardboiled egg, minced, can be substituted).
- 4. An artificial sea salts mixture for salt-water aquaria, such as "Instant Ocean" (available at any local aquarium store). This mixture of salts will work best as it contains all of the salts found in seawater, but, in a pinch, non-iodized table salt will do.
- 5. Sand (from local beach or store).
- 6. Tap water source.
- 7. 1/3-liter (per student) "microbial mud" sample gathered from a local aquatic environment.

  Look for mud that has a green "tint" on the surface with dark black mud directly underneath

the surface (see pictures below"). Amount will vary depending on the size of plastic container. Snails and other critters are fun to collect but may not survive in a microbial mat nor in the classroom. Some suggestions for aquatic environments with the "right kinds" of microbes include:

- "Green mud" at the highest point of a tidal zone, during low tide, or the area of land where water does not cover tidal zone for most of the day.
- Estuarine and marsh environments (refer to "Estuarine Microbial Mat Specimen under Procedure"" below for specific modifications). For gathering specimens at a marsh location: look for a green tint on the surface of mud.



Algae grow in a variety of familiar locations. They may not make mats where you collect them, but they might in the lab.

- o Green or brown surface sediments in local rivers, or streams.
- Green coverings of sidewalks, rain gutters, some water fountains
- Green slime in fish tanks

Note: There is no minimum sample size. You can grow any amount of sample size on top of sand.

# Lab and Field Safety

Microbes and microbial mat communities consist of a combination of harmless bacteria and algae. However, when sampling microbial mats from the outdoor environment, you should always consider the possibility that non-point source pollution could occur in that area. Students should always wash their hands after handling microbial mats or "mud." Never eat during the lab. Goggles should also be worn during the lab.

### **Procedure**

The following procedure assumes a box about 6 cm high and about 3 cm on a side. You can use pretty much any kind of container. Adapt the quantities of mud, sand, etc. below to your container.

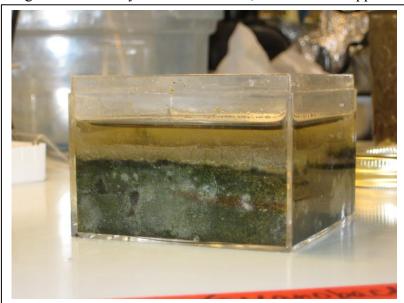
- 1. Draw a line with a permanent marker around a box 2.5 cm and a line at 5 cm from the bottom.
- 2. Weigh out .5 g of Plaster of Paris (provides a sulfur source for some bacteria).
- 3. Tear up a 2.5 cm<sup>2</sup> piece of paper (provides additional carbon for some bacteria).

- 4. Place Plaster of Paris and the torn paper into the bottom of a box. (Be careful to make sure the Plaster of Paris is only on the bottom of the box and not covering the sides. The bacteria that use sulfur as a food source grow best in an environment with low oxygen).
- 5. Fill the box to the 2.5 cm line with soil and/or mud.
- 6. Tap the box gently on the table to remove any air bubbles.
- 7. Add water to the 5 cm line
- 8. Students should write their initials and period on both the cover and one side of the box.
- 9. Tape the caps askew on the boxes using 1 piece of tape on 1 side of the cover.
- 10. Place the boxes near a sunny window
- 11. In 3-4 weeks you should see layers of different colors indicating the growth of different kinds of bacteria and algae.

## **Care and Feeding of Your Microbial Mat**

Use a permanent marker and draw a short horizontal line on the outside of your container, indicating the water line. As your microbial mat is growing you may notice the water evaporating. If you do, add water up to your water line. To avoid disturbing the surface of your microbial mat, use a water dropper

to slowly add water over the top of the microbial mat. Clean off any mud that may have gotten onto the inside or outside of your plastic container. This will ensure that light is able to enter the sides of your container at the top, allowing photosynthetic organisms to grow. It may help to rotate the container every few days. ALWAYS USE FRESH WATER WHEN REHYDRATING and NEVER POUR OUT WATER FROM YOUR MAT (this may interfere with the osmolarity, especially with ocean microbial mats)! Degradation of organic matter in the deeper parts of the mat is generally enough to supply the top layers with sufficient nutrients, but you could supply a very small amount of fertilizer if you notice the previously green layer starting to fade.



Constructed microbial mat in a plastic box. The microbes have created layers with their metabolic processes.- Photo by Allison Pfeifer